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(54) Title: DIETETIC SOY BASED PRODUCT, METHOD FOR PRODUCTION THEREOF AND USE THEREOF

(57) Abstract

The dietetic soy based product exhibits the following composition of fat free dry matter: proteins (N x 6.25) > 50 %, soluble dietary fibres > 5 %, phytate < 0.3 %, stachyose < 0.4 %, raffinose < 0.4 %. This dietetic soy based product contains only negligible amounts of phytate, stachyose and raffinose, it contains soluble fibres in large amounts, it is relatively cheap and exhibits good organoleptic properties.

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DIETETIC SOY BASED PRODUCT, METHOD FOR PRODUCTION THEREOF AND USE THEREOF

The invention comprises a dietetic soy based product comprising proteins and carbohydrates, a method for production thereof, and a use thereof.

Dietetic soy based products comprising proteins and carbohydrates belong to the prior art. Examples are soy milk, soy isolate and soy concentrate. Such dietetic soy based products can be used as nutrients or as part of nutrients both intended for humans and animals. If used as a part of nutrients, e.g. fat and/or vitamins can be added in order to produce a full nutrient.

The dietetic soy based product soy milk is a well known dietetic product for human nutrition, which can be produced from many different soy raw materials as starting materials, *vide* W. Shurtleff & A. Aoyagi, 1979, Tofu and soy milk production, The book of tofu, volume II. ISBN 0-933332-01-7. If full fat soy flour or defatted soy flour are used as starting materials the soy milk will contain high amounts of phytate, stachyose and raffinose, and the soy milk will contain almost no dietary fibres. A method for improving the taste and compositional quality of soy milk is utilization of soy protein concentrate as starting material in which the soluble oligosaccharide fraction including stachyose and raffinose has been eliminated. However, phytate will be present in relatively high amounts and the price of the product will be high due to the more expensive starting material and due to the cost price of the dietary fibre fraction that has to be added separately. Alternatively a soy protein isolate can be used as a starting material. However, also in this case phytate will not be substantially eliminated, and soy protein isolate is a high price raw material.

In regard to the significance of phytate, stachyose, raffinose and dietary fibres the following can be noted.

Methods for eliminating phytate are described in the prior art. Absence of phytate is wanted as phytate binds the minerals in foods resulting in a decrease in mineral uptake. US Patent No. 5,248,804 describes the elimination of phytate by use of an ion exchange process. The soy protein free from phytate is claimed useful as a nutrient for humans. However, such a process will be relatively costly due to

loss in yield and costs for regeneration of ion exchange materials. WO 90/08476 describes the production of a low-phytate soy protein isolate or concentrate by use of a phytate degrading enzyme. This product, however, will contain relatively high amounts of oligosaccharides, which are not digestible in the human intestine. In US Patent No. 3,297,548 the use of phytase for addition to the feeds for animals has been described showing how the mineral uptake is improved by decreasing phytate in the feed.

The fraction of soluble carbohydrates in soy comprises oligosaccharides which are not digestible in the human intestine, i.e. stachyose and raffinose. Fermentation of these oligosaccharides in the intestine leads to flatulence. One way of partly eliminating these oligosaccharides in a dietetic soy based product is the use of more expensive starting materials, such as soy protein concentrate. Also, they can be totally eliminated by use of soy protein isolate as the starting material. Recently an enzymatic method for reduction of these carbohydrates has been described, *vide* European Patent No. 0479596. α-galactosidase is used for hydrolysis of stachyose and raffinose.

The dietary fibre fraction (the plant cell wall polysaccharide fraction) exhibits a beneficial influence on the intestine. However, a problem exists in formulating a dietetic soy based produced with the dietary fibre fraction from soy as it has a high water binding capacity leading to high viscous formulations which limit the concentration of fibres.

Thus, the purpose of the invention is the provision of a dietetic soy based product of the above indicated kind, which contains only negligible amounts of phytate, stachyose and raffinose, which contains soluble dietary fibres in large amounts, which is relatively cheap, and which exhibits good organoleptic properties, a method for production thereof, and a use thereof.

The dietetic soy based product according to the invention comprising proteins and carbohydrates is characterized by the fact that the dietetic soy based product exhibits the following composition of fat free dry matter:

Proteins (N x 6.25)	> 50%
Soluble dietary fibres	> 5%
Phytate	< 0.3%
Stachyose	< 0.4%
Raffinose	< 0.4%

Surprisingly it has been found that the dietetic soy based product according to the invention fulfils the purpose of the invention, as it contains negligible amounts of phytate, stachyose and raffinose, as it contains soluble dietary fibres in large amounts and as it surprisingly is relatively cheap, and exhibits good organoleptic properties.

Also the invention comprises a method for production of the dietetic soy product according to the invention, and the method is characterized by the fact that

a) a soy based raw material is suspended in water
b) the following enzymes are added to the suspension:
    1) phytase in an amount sufficient for reduction of the content of phytate to below 50% of the initial content, preferably below 10% of the initial content.
    2) alfa-galactosidase in an amount sufficient for reduction of the content of stachyose and raffinose to below 50% of the initial content, preferably below 10% of the initial content.
    3) plant cell wall polysaccharide solubilizing enzymes in an amount sufficient for solubilizing pectic substances in an amount above 10% of the insoluble carbohydrate fraction of the soy based raw material, and
c) the thus formed, enzyme treated suspension is heat treated in order to inactivate the enzymes.

In this specification with claims phytase is to be understood as any enzyme, which is able to remove at least one phosphate group from phytate.

In this specification with claims α-galactosidase is to be understood as any enzyme which is able to produce galactose monomers from oligomers

containing α-linked galactose. The α-galactosidase activity determination method is described in the publication AF 204, which on request can be obtained from Novo Nordisk A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark. The principle of AF 204 comprises that α-galactosidase (α-D-galactosidase-galactohydrolase, EC 3.2.1.22) hydrolyzes the colourless p-nitrophenyl-α-D-galactopyranosidase (p-NPGAL) forming 4-nitrophenol, which is yellow in alkaline solution.

In this specification with claims pectic substances are to be understood as polymers comprising α-linked galacturonic acid in the main chain.

Even if some of the process steps (e.g. the treatment with phytase and the treatment with α-galactosidase) belongs to the prior art in another context, the combination of the above indicated steps comprising the specified enzyme treatments and the final heat treatment, is novel and generates a surprising effect, i.e. the formation of the dietetic soy based product according to the invention with the above indicated surprising characteristics.

In regard to step a) it is to be understood that the following materials can be used as soy based raw materials: defatted soy flour, full fat soy flour, soy flakes, and soy concentrate. More generally it can be stated that the soy based raw material which is the starting material in the method according to the invention, can be defined as comprising the constituents of the soy bean which are provided without any further processing than dehulling and grinding the whole soy bean. A heat treatment of the dehulled and ground bean does not limit the use of the soy based raw material although a soy based raw material which has only been exposed to a limited heat treatment is preferred. The soy based raw material can also be defatted soy flakes or defatted soy flour as these soy based raw materials are available at very low cost. It could be an advantage also to use defatted soy flakes or defatted soy flour, as the absence of oil facilitates the method according to the invention. Removing the oil in an extraction process and refining it in a traditional way also results in a more bland tasting oil which can then be added to the final product when formulating the dietetic soy based product according to the invention. It is to be understood that also soy protein concentrate and soy protein isolate can be used as the soy based raw material in the process according to the invention. However, due to the price of this kind of raw materials the dietetic soy based

product according to the invention appearing as the end product will be more expensive and lack some of the wanted soy components. Soy protein concentrate contains only very low amounts of soluble sugars and also exhibits a low content of non-degradable carbohydrates. Thus, if soy protein concentrate is used as the soy based raw material in the method according to the invention dietary fibres will have to be added to the end product, and if soy protein isolate is used as the soy based raw material in the method according to the invention, soluble sugars and soluble components from the fibre fraction will have to be added to the end product.

In regard to step b) it is to be understood that the enzymes can be added in any order or simultaneously, and that pH and temperature of the suspension should be adjusted in such a manner that the enzymes exhibit a reasonable activity and stability. If the pH should be lowered, and an acid is used for pH adjustment, such acid should be chosen among food grade mineral acids and organic acids or combinations hereof, examples being hydrochloric acid, phosphoric acid, citric acid, lactic acid, glucone-delta-lactone, acetic acid, and malic acid, however, the pH can also be lowered by fermentation with pH lowering microorganisms. Furthermore, in regard to the plant cell wall solubilizing enzymes it is to be understood that these comprise enzymes, which can degrade the pectic substances of soy to soluble high molecular weight molecules, which appear as dietary fibres when analyzed by the method described in AOAC 15 (1990) 985.29, whereby the total fibre content is corrected for protein. The plant cell wall solubilizing enzymes should be added in an amount which is able to solubilize a significant part, i.e. above around 10%, of the pectic substances. A supplementary advantage in relation to the method according to the invention is the fact that it is possible to perform the entire enzyme addition step b) at the same pH, i.e. around 6.0, due to the fact that it is possible to select workable types of the three kinds of enzymes, which exhibit a good compromise in regard to activity and stability at a pH value around 6.0.

In regard to step c) it is to be understood that the heat treatment should not be carried out to such an extent that valuable parts of the formed dietetic soy based product are decomposed.

In regard to all the enzymes used in the method according to the invention it is to be understood that their origin is immaterial; thus, they may be produced by means of a microorganism, which exhibits a natural ability to produce the enzyme in question, or they may be produced by genetic engineering.

A preferred embodiment of the method according to the invention is characterized by the fact that the soy based raw material is defatted soy flakes or defatted soy flour. In this case the oil from the bean can be utilized separately and the method according to the invention will become facilitated. Also an extraction of the oil and a subsequent processing of the oil in conventional processes will lead to an organoleptically better soy oil which can then be added to the final dietetic soy based product if wanted.

A preferred embodiment of the method according to the invention is characterized by the fact that the soy based raw material is a soy material with a nitrogen solubility index above 50%. The nitrogen solubility index (NSI) is defined in A.O.C.S. Method Ba 11-65, revised 1969. In this case the soy based raw material has been exposed to a low heat treatment only, and the final dietetic soy based product is fully soluble and is obtained in high yield.

A preferred embodiment of the method according to the invention is characterized by the fact that the phytase is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus niger*. In this manner a phytase preparation with a high phytase activity can be used, and furthermore, an *Aspergillus niger* phytase preparation is available on the market.

A preferred embodiment of the method according to the invention is characterized by the fact that the alfa-galactosidase is producible by means of a microorganism belonging to the genera *Aspergillus*, *Trichoderma*, or *Penicillium*, preferably to the species *Aspergillus niger*, reference being made to WO 94/23022. In this manner an α-galactosidase preparation with a high α-galactosidase activity can be used, and furthermore corresponding α-galactosidase preparations are available on the market.

A preferred embodiment of the method according to the invention is characterized by the fact that the plant cell wall solubilizing enzymes comprise a

polygalacturonase, which is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus aculeatus*, reference being made to WO 94/14952. In this manner a polygalacturonase with a high polygalacturonase activity can be used.

A preferred embodiment of the method according to the invention is characterized by the fact that the plant cell wall solubilizing enzymes comprise a pectin methyl esterase, which is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus aculeatus*, reference being made to WO 94/25575. When this enzyme is used together with polygalacturonase an increase in solubilization of pectic substances/fibres is obtained.

A preferred embodiment of the method according to the invention is characterized by the fact that the plant cell wall solubilizing enzymes comprise a rhamnogalacturonase, which is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus aculeatus*, reference being made to WO 92/19728 and WO 94/20612. In this manner a rhamnogalacturonase with a high rhamnogalacturonase activity can be used.

A preferred embodiment of the method according to the invention is characterized by the fact that the plant cell wall solubilizing enzymes comprise a rhamnogalacturonan acetyl esterase, which is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus aculeatus*, reference being made to WO 93/20190. When this enzyme is used together with rhamnogalacturonase an increase in solubilization of pectic substances/fibres is obtained.

A preferred embodiment of the method according to the invention is characterized by the fact that the effluent from step b) is separated in a soluble and an insoluble fraction, that the insoluble fraction is discarded, and that the soluble fraction is further processed as indicated in step c). In this embodiment the final dietetic soy based product is soluble, thus making it easier to use the product as a constituent in a nutrient. Furthermore the organoleptic properties are improved, due to the fact that the insoluble fraction is eliminated.

A preferred embodiment of the method according to the invention is characterized by the fact that the pH of the effluent from step b) is adjusted to 7 - 9, preferably to 7.5 - 8.5, that the pH adjusted effluent is subsequently separated in a soluble and an insoluble fraction, that the insoluble fraction is discarded and that the soluble fraction is further processed as indicated in step c). In this embodiment the protein solubility of the final product is increased, and consequently the yield is increased.

A preferred embodiment of the method according to the invention is characterized by the fact that the heat treatment in step c) is carried out as a UHT treatment and a cooling in a flash process. By flash cooling off-flavors are removed, whereby a dietetic soy based product with better organoleptic properties is provided. The UHT treatment comprising a direct heating by mixing with steam under pressure secures an instant heating. Instant heating and instant cooling allows a more well-defined heat treatment and makes it easier to keep proteins and other components soluble. The holding time should be long enough to secure inactivation of the enzymes. Temperatures in the range of 120°C to 145°C and holding times from 3 to 30 seconds are preferred. However, it is to be understood that also indirectly heat treatment even at low temperatures down to 70°C and a corresponding longer holding time can be used, although the solubility and taste quality of the dietetic soy based product will decrease.

A preferred embodiment of the method according to the invention is characterized by the fact that the effluent from step c) is concentrated by nanofiltration by means of a salt permeable membrane, and that the permeate is discarded. The final dietetic soy product is the retentate. This embodiment allows production of the dietetic soy based product according to the invention as a concentrate, which simply and cheaply can be converted to a powder by spray drying, which powder in its turn can easily be stored and distributed. The concentration can also be performed by reverse osmosis or evaporation.

Also, the invention comprises a use of the dietetic soy based product according to the invention, as a nutrient or part of a nutrient, intended for humans and/or animals.

The invention will be illustrated by the following example.

EXAMPLE 1

62.8 g of defatted soy flour with high nitrogen solubility index (Cargil 200/90) was suspended in 337.2 g of water at 50°C resulting in a protein concentration of 8%.

pH was adjusted to 6.0 by means of 6N HCl.

The mixture was divided into 2 lots:

1. Addition of enzymes as indicated below and reaction for 4 hours at 50°C.

α-galactosidase (Novo Nordisk SP415). Concentration 0.4% of the soy flour.

Phytase (Novo Nordisk PPQ 4415). Concentration 0.2% of the protein content.

Rhamnogalacturonase (Novo Nordisk SP568). Concentration 0.1% of the protein content.

2. Blank without enzyme addition. 4 hours at 50°C.

After 4 hours both lots were adjusted to pH 8.0 by means of 4N NaOH. After centrifugation, the supernatant was pasteurized and freeze dried.

Analysis of dried products:

	Lot 1	Lot 2
Dry matter, %	95.2	98.2
Protein, %	59.2	60.2
Dietary fibre, %	5.7	2.5
Stachyose, %	0.36	5.30
Raffinose, %	0.20	1.40
Phytate, %	0.16	1.54

The content of stachyose and raffinose was determined by an HPLC method which included separation of the oligosaccharides on a Dionex CarboPac PA column and Pulsed Amperometric Detection as described by the Dionex Corporation (Sunnyval, CA) in: Analysis of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection (Dionex Technical Note TN20).

The taste of the sample from lot 1 was described as more bland with less pronounced soy flavor and with a sweeter taste compared to the sample from lot 2.

CLAIMS

1. Dietetic soy based product comprising proteins and carbohydrates, characterized by the fact that the dietetic soy based product exhibits the following composition of fat free dry matter:

Proteins (N x 6.25)	> 50%
Soluble dietary fibres	> 5%
Phytate	< 0.3%
Stachyose	< 0.4%
Raffinose	< 0.4%

2. Method for production of the dietetic soy product according to Claim 1, characterized by the fact that

a) a soy based raw material is suspended in water
b) the following enzymes are added to the suspension:
   1) phytase in an amount sufficient for reduction of the content of phytate to below 50% of the initial content, preferably below 10% of the initial content.
   2) alfa-galactosidase in an amount sufficient for reduction of the content of stachyose and raffinose to below 50% of the initial content, preferably below 10% of the initial content
   3) plant cell wall polysaccharide solubilizing enzymes in an amount sufficient for solubilizing pectic substances in an amount above 10% of the insoluble carbohydrate fraction of the soy based raw material, and
c) the thus formed, enzyme treated suspension is heat treated in order to inactivate the enzymes.

3. Method according to Claim 2, characterized by the fact that the soy based raw material is defatted soy flakes or defatted soy flour.

4. Method according to Claim 2, characterized by the fact that the soy based raw material is a soy material with a nitrogen solubility index above 50%.

5. Method according to Claims 2 - 4, characterized by the fact that the phytase is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus niger*.

6. Method according to Claims 2 - 5, characterized by the fact that the alfa-galactosidase is producible by means of a microorganism belonging to the genera *Aspergillus*, *Trichoderma*, or *Penicillium*, preferably to the species *Aspergillus niger*.

7. Method according to Claims 2 - 6, characterized by the fact that the plant cell wall solubilizing enzymes comprise an polygalacturonase, which is producible by means of a microorganism belonging to the genes *Aspergillus*, preferably to the species *Aspergillus aculeatus*.

8. Method according to Claims 2 - 7, characterized by the fact that the plant cell wall solubilizing enzymes comprise a pectin methyl esterase, which is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus aculeatus*.

9. Method according to Claims 2 - 8, characterized by the fact that the plant cell wall solubilizing enzymes comprise a rhamnogalacturonase, which is producible by means of a microorganism belonging to the genes *Aspergillus*, preferably to the species *Aspergillus aculeatus*.

10. Method according to Claims 2 - 9, characterized by the fact that the plant cell wall solubilizing enzymes comprise a rhamnogalacturonan acetyl esterase, which is producible by means of a microorganism belonging to the genus *Aspergillus*, preferably to the species *Aspergillus aculeatus*.

11. Method according to Claims 2 - 10, characterized by the fact that the effluent from step b) is separated in a soluble and an insoluble fraction, that the insoluble fraction is discarded, and that the soluble fraction is further processed as indicated in step c).

12. Method according to Claim 11, characterized by the fact that the pH of the effluent from step b) before the separation is adjusted to 7 - 9, preferably 7.5 - 8.5.

13. Method according to Claims 2 - 12, characterized by the fact that the heat treatment in step c) is carried out as a UHT treatment and a cooling in a flash process.

14. Method according to Claims 2 - 13, characterized by the fact that the effluent from step c) is concentrated by nanofiltration by means of a salt permeable membrane, and that the permeate is discarded.

15. Use of the dietetic soy based product according to Claim 1 as a nutrient or part of a nutrient, intended for humans and/or animals.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23J 1/14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23J, A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EDOC, WPI, DIALINDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 9008476 (ALKO LIMITED), 9 August 1990 (09.08.90), claims 1-3,22-23 --	1-15
A	EP, A1, 0479596 (CARGILL B.V.), 8 April 1992 (08.04.92), claims 1,3,14-15 -- --------	1-15

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9008476	09/08/90	CN-A- 1048310	09/01/91
		EP-A,A,A 0380343	01/08/90
		JP-T- 4503002	04/06/92
EP-A1- 0479596	08/04/92	CA-A- 2052608	04/04/92
		US-A- 5100679	31/03/92